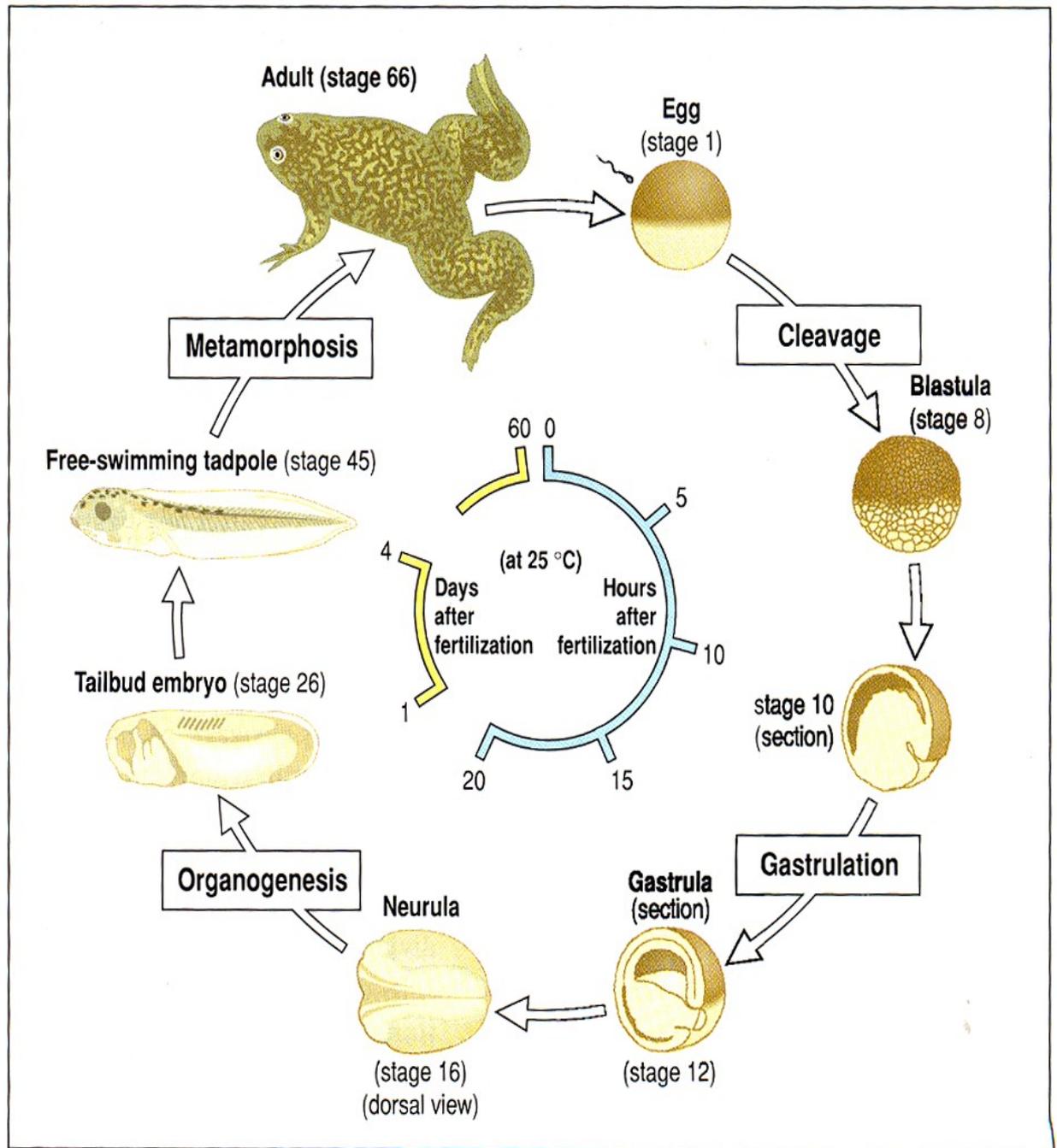
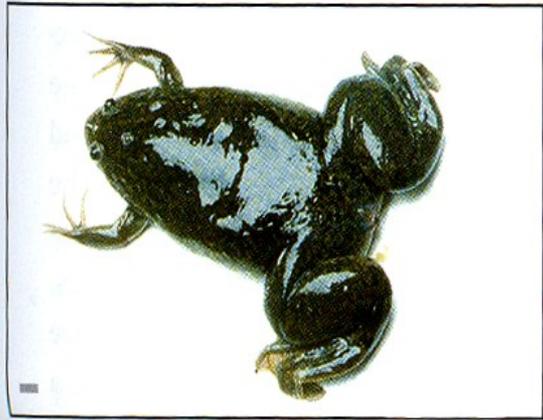
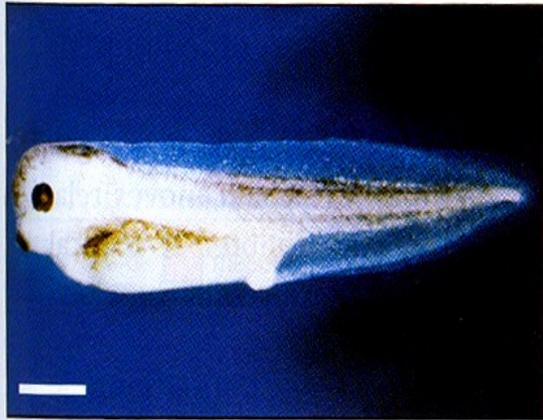
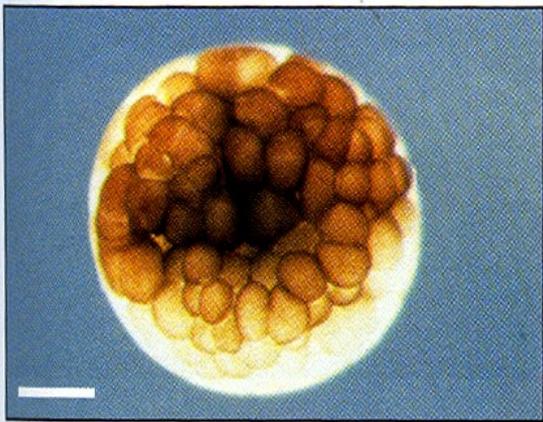


MATERNAL mRNA LOCALIZATION IN THE DEVELOPMENT

Putting RNAs at the right place in the right time



GASTRULATION/NEURULATION

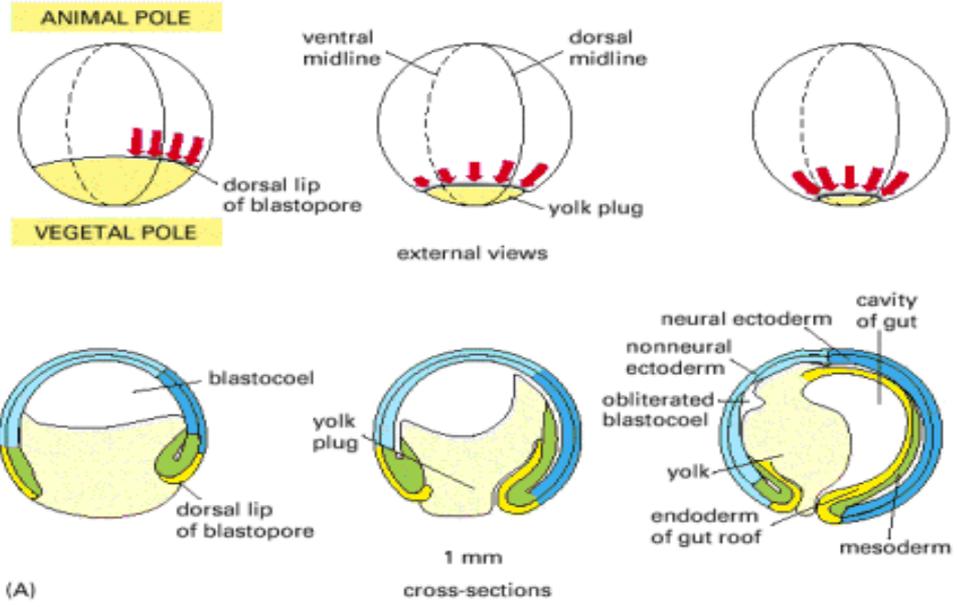
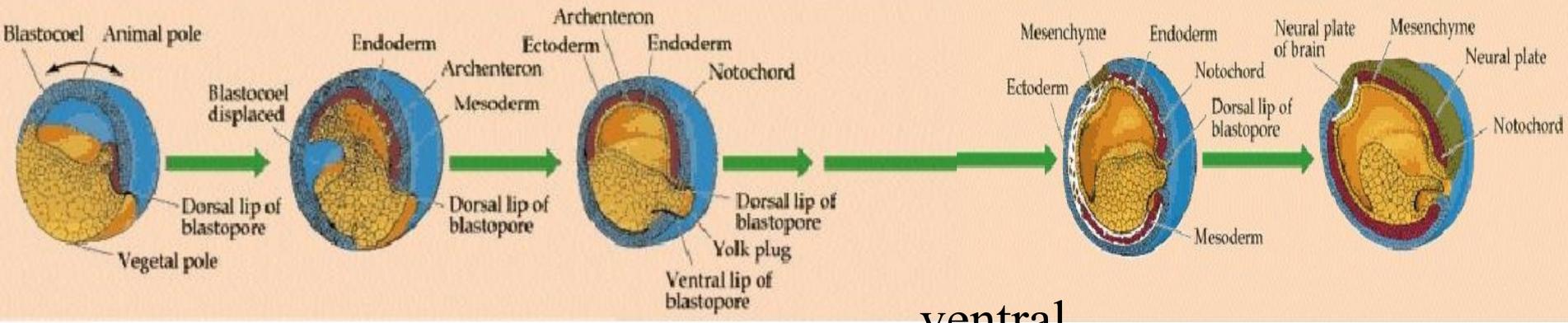
1 Gastrulation begins when cells just below the center of the gray crescent invaginate to form the dorsal lip of the future blastopore.

2 Cells of the animal pole spread out, pushing surface cells below them toward and across the dorsal lip. Those cells involute into the interior of the embryo, where they form the endoderm and mesoderm.

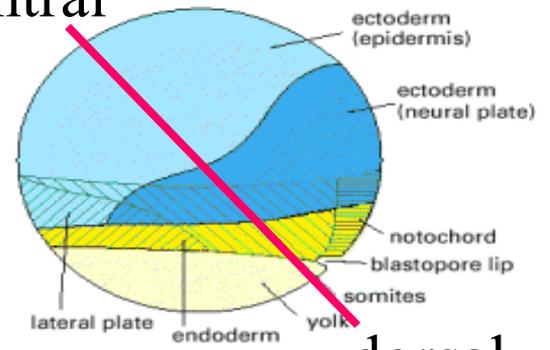
3 This involution creates the archenteron and destroys the blastocoel. The dorsal lip forms a circle, with cells moving to the interior all around the blastopore; the yolk plug is visible through the blastopore.

4 Continued development gives rise to a notochord derived from mesoderm.

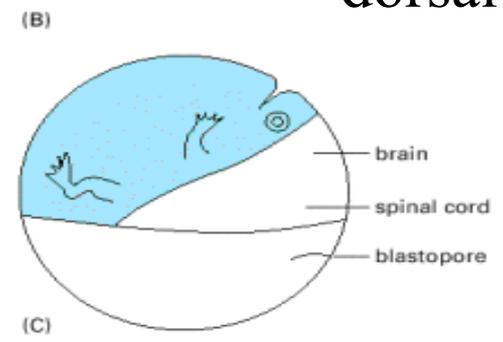
5 The beginnings of the nervous system (green) are derived from ectoderm.



ventral



dorsal



(C)

ACCURACY AND REPRODUCIBILITY IN ACQUIRING THE CELLULAR FATE WITHIN THE EMBRYO

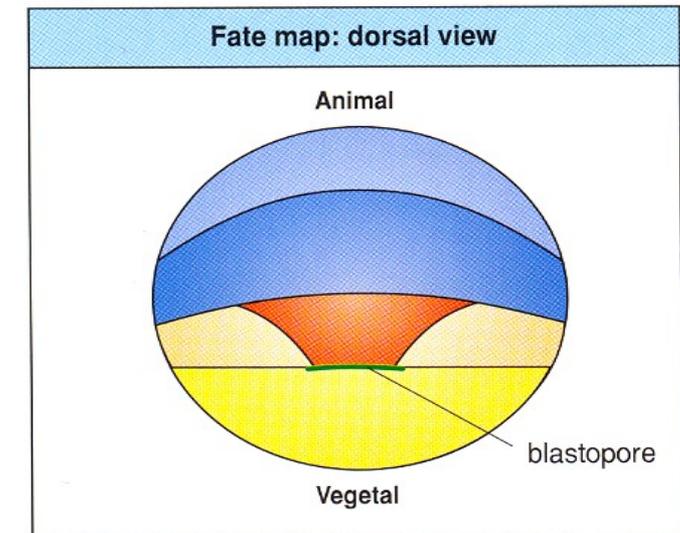
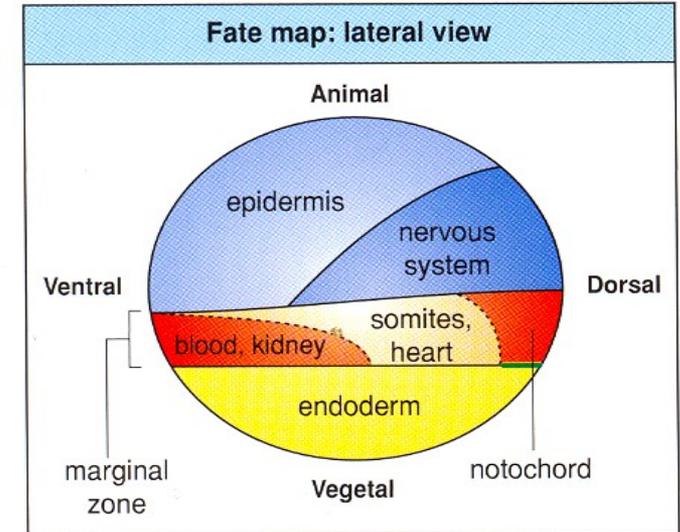
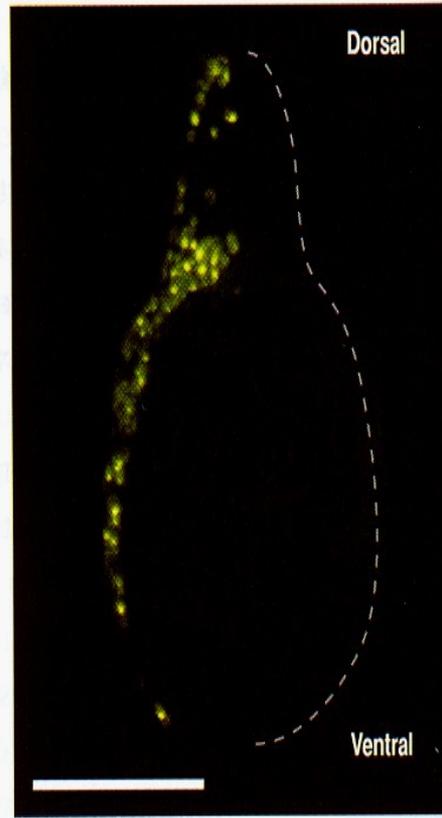
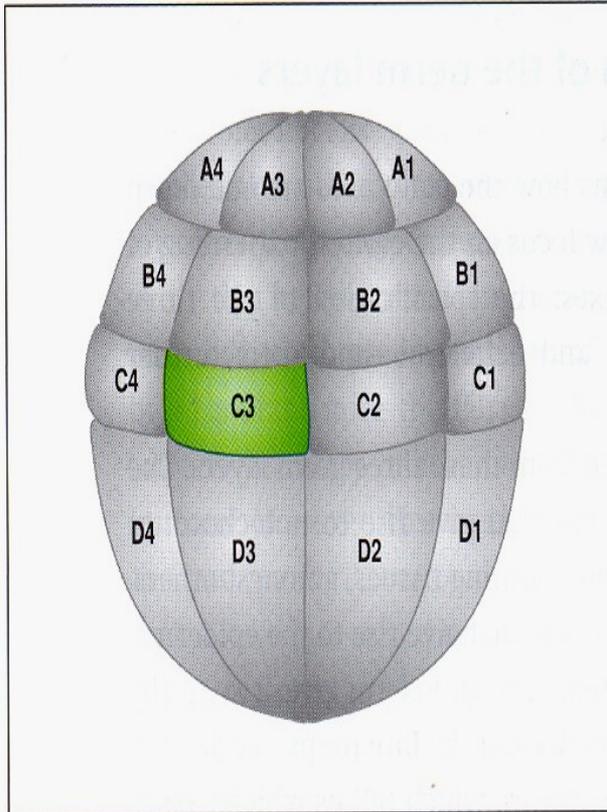
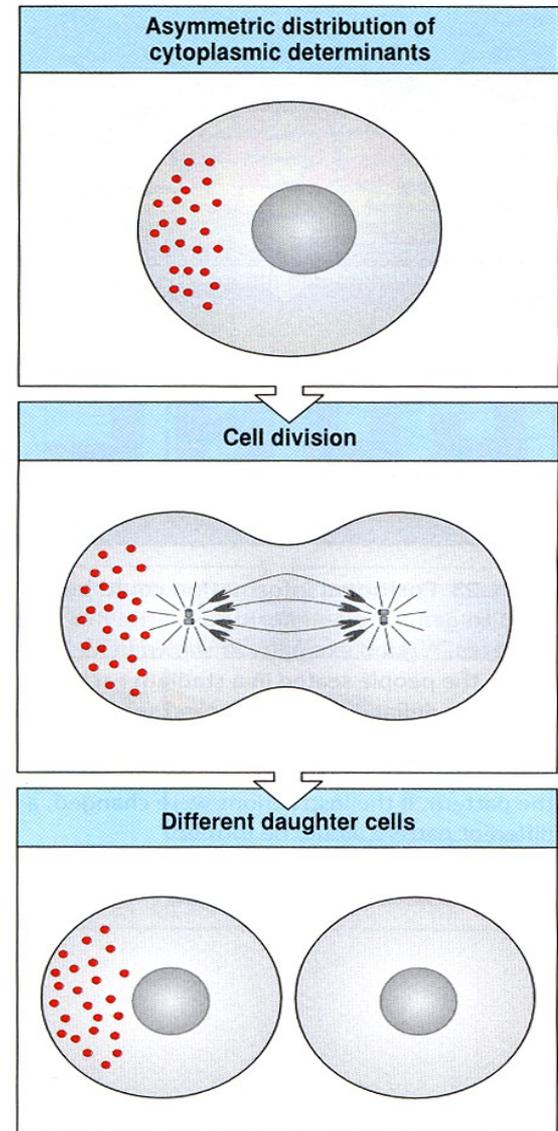


Fig. 3.18 Fate map of a late *Xenopus* blastula.

The vegetal pole gives rise to the epidermis and

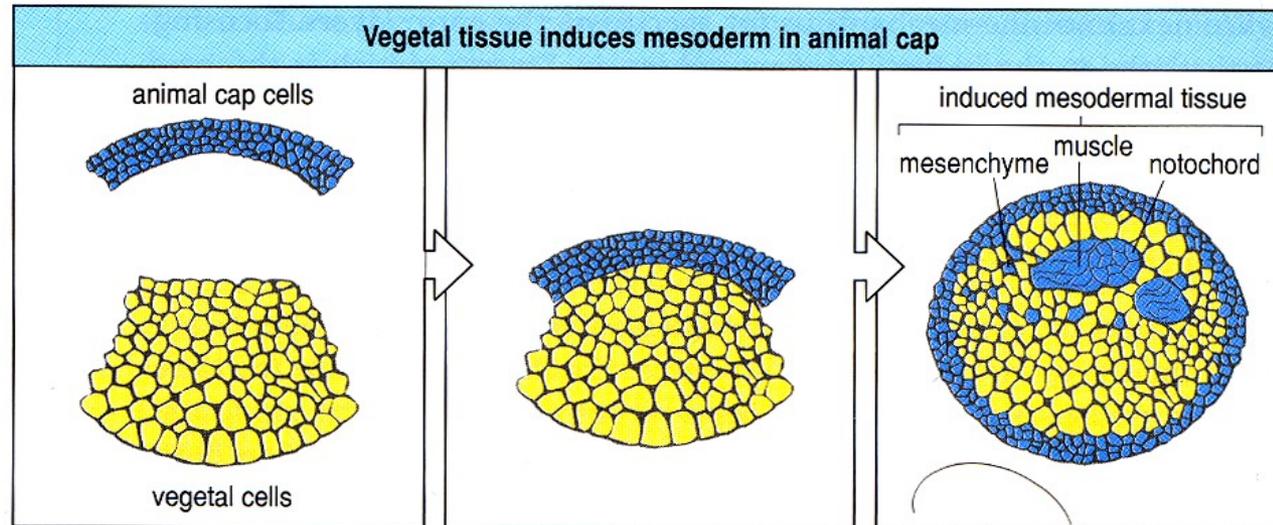
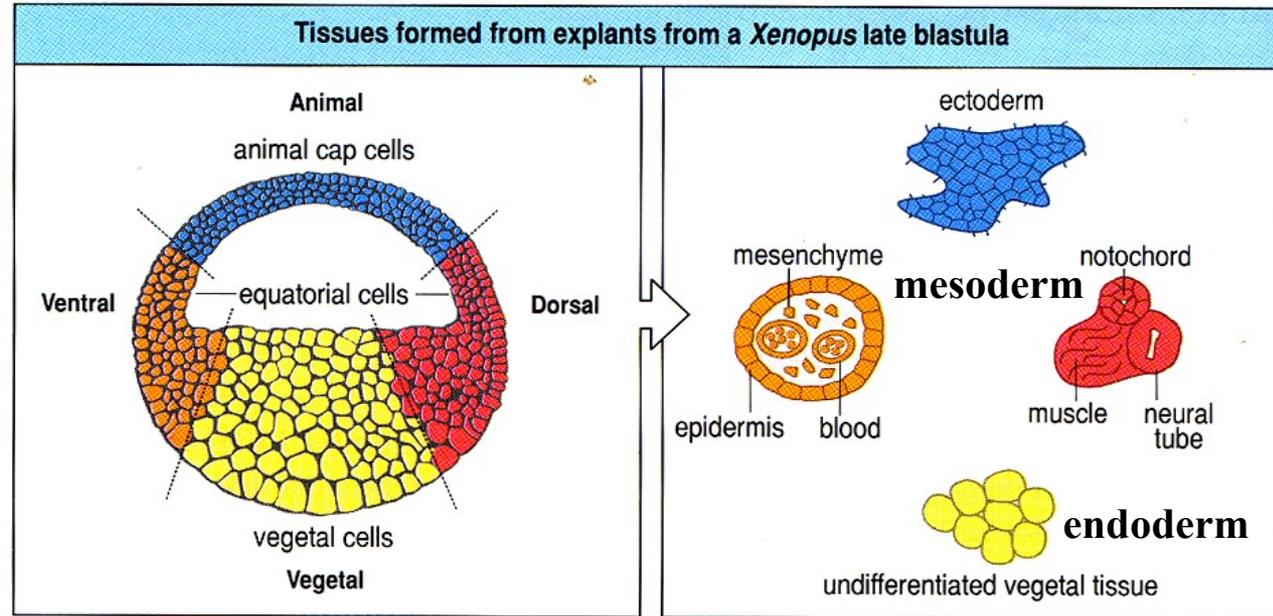
Differential mRNA localization to subcellular compartments

- allows for spatial regulation of gene expression
- essential for polarity set-up in oogenesis
- patterning during embryogenesis
- in *Xenopus*: localized maternal mRNAs generate developmental polarity along the animal/vegetal axis.



CELL-TO-CELL SIGNALING vs. MATERNAL FACTORS IN TISSUE SPECIFICATION

Ectoderm and endoderm are specified by maternal factors in the egg
Mesoderm is induced by vegetal tissue



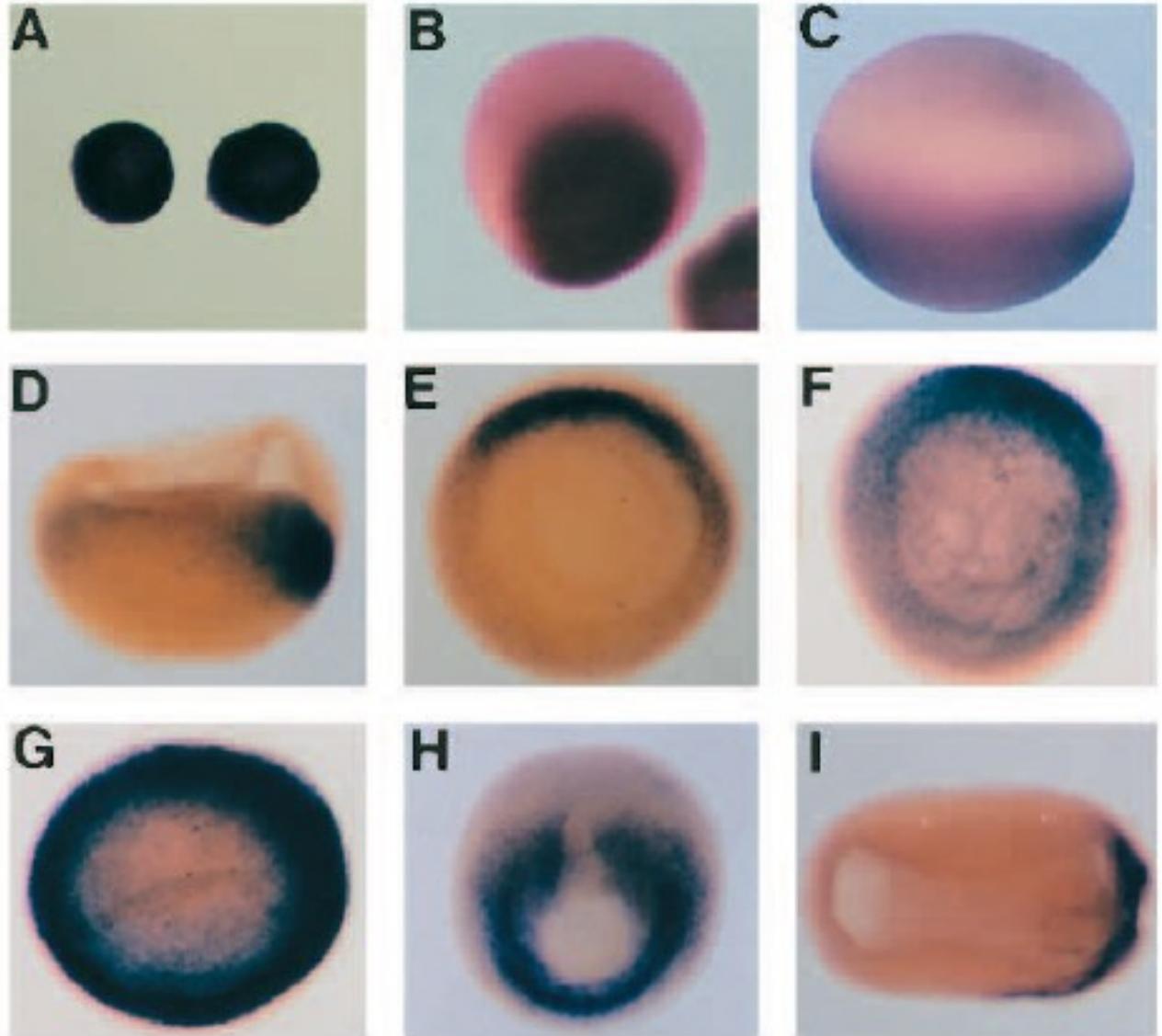
MATERNAL vs. ZYGOTIC REGULATORS

Summary: genes involved in patterning of axes and germ layers

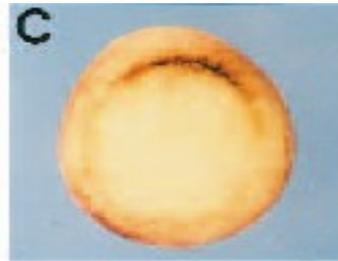
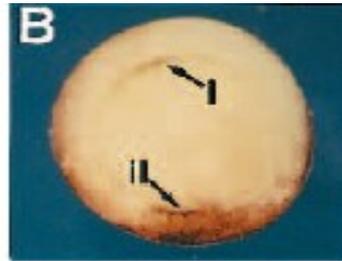
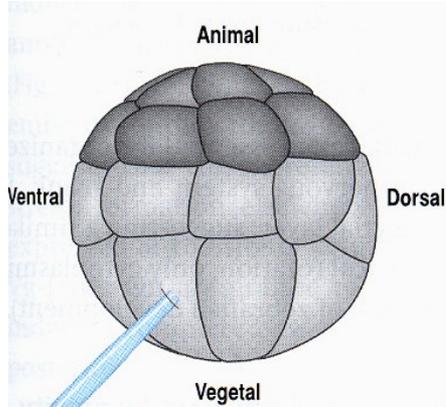
Gene	Maternal/ Zygotic	Type of protein	Where expressed	Effects
<i>activin</i>	Z	TGF- β family	?	mesoderm induction
<i>BMP-4</i>	Z	transcription factor	late blastula	ventralizes mesoderm
<i>Brachyury</i>	Z	transcription factor	early mesoderm	mesoderm development
β -catenin	M	gene regulatory protein	egg	dorsalizing signal
<i>cerberus</i>	Z	secreted	vegetal egg	mesoderm inhibition
<i>chordin</i>	Z	secreted signal molecule	organizer	dorsalizes mesoderm
<i>derriere</i>	Z	TGF- β family	vegetal egg	mesoderm induction
<i>fibroblast growth factor</i>	Z	secreted signal molecule	blastula	ventral mesoderm induction
<i>gooseoid</i>	Z	transcription factor	organizer	organizer function
<i>GSK-3</i>	M	protein kinase	egg	suppresses dorsalizing signals
<i>HNF-3β</i>	Z	transcription factor	organizer	organizer development
<i>noggin</i>	M/Z	secreted	organizer	dorsalizes mesoderm
<i>Pintallavis</i>	Z	transcription factor	organizer	?
<i>siamois</i>	Z	transcription factor	dorsal blastula	dorsalizing signal
<i>VegT</i>	M	transcription factor	vegetal egg	induces endoderm and mesoderm signals
<i>Vg-1</i>	M	TGF- β family	vegetal egg	mesoderm induction
<i>Xlim-1</i>	Z	transcription factor	organizer	?
<i>Xnot</i>	Z	transcription factor	organizer	notochord specification
<i>Xnr-1</i>	Z	secreted	vegetal egg	mesoderm induction
<i>Xnr-2</i>	Z	secreted	vegetal egg	mesoderm induction
<i>Xnr-4</i>	Z	secreted	vegetal egg	mesoderm induction
<i>Xwnt-11</i>	M	Wnt family	vegetal egg	mesoderm induction
<i>Xwnt-8</i>	Z	Wnt family	propective mesoderm	ventralizes mesoderm

VegT (T-box family transcription factor)

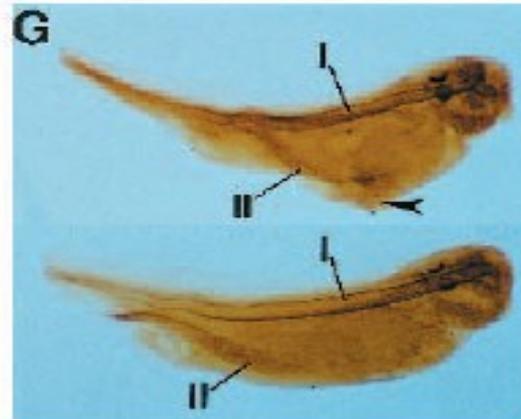
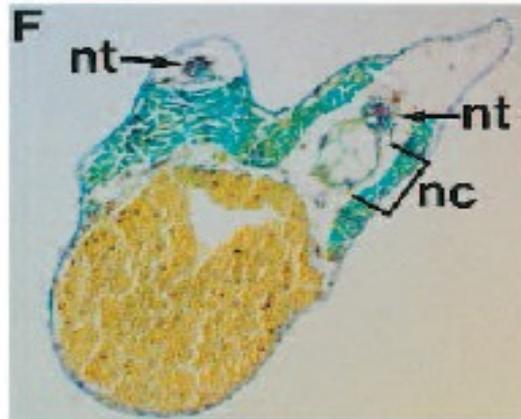
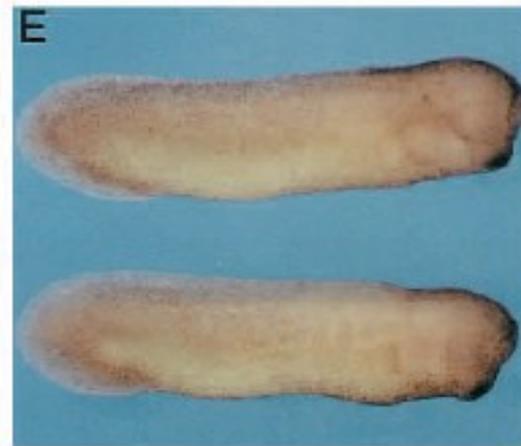
- A** – stage I oocytes
- B** – stage IV oocytes
- C** – ovulated egg
- D** – stage 9.5 embryo
- E** – stage 9.5 embryo
(vegetal pole view)
- F** – stage 10.25 embryo
(vegetal pole view)
- G** – stage 10.5 embryo
(vegetal view)
- H** – stage 12.5 embryo
(posterior view)
- I** – mid neural fold
embryo (stage 16)



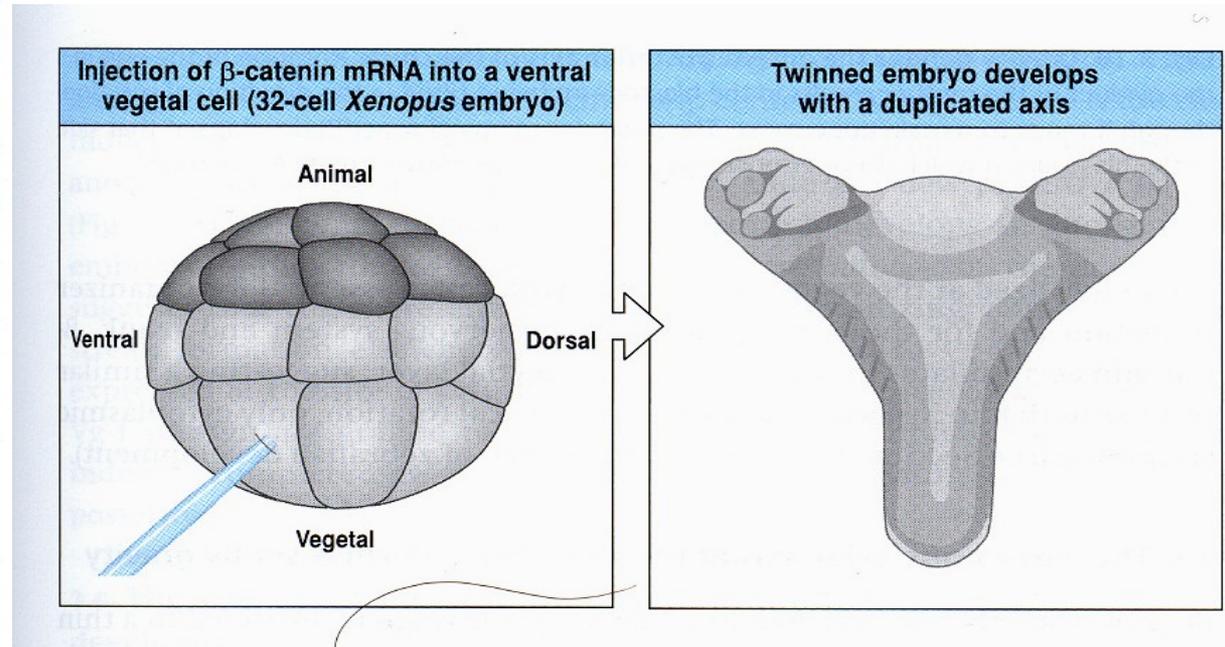
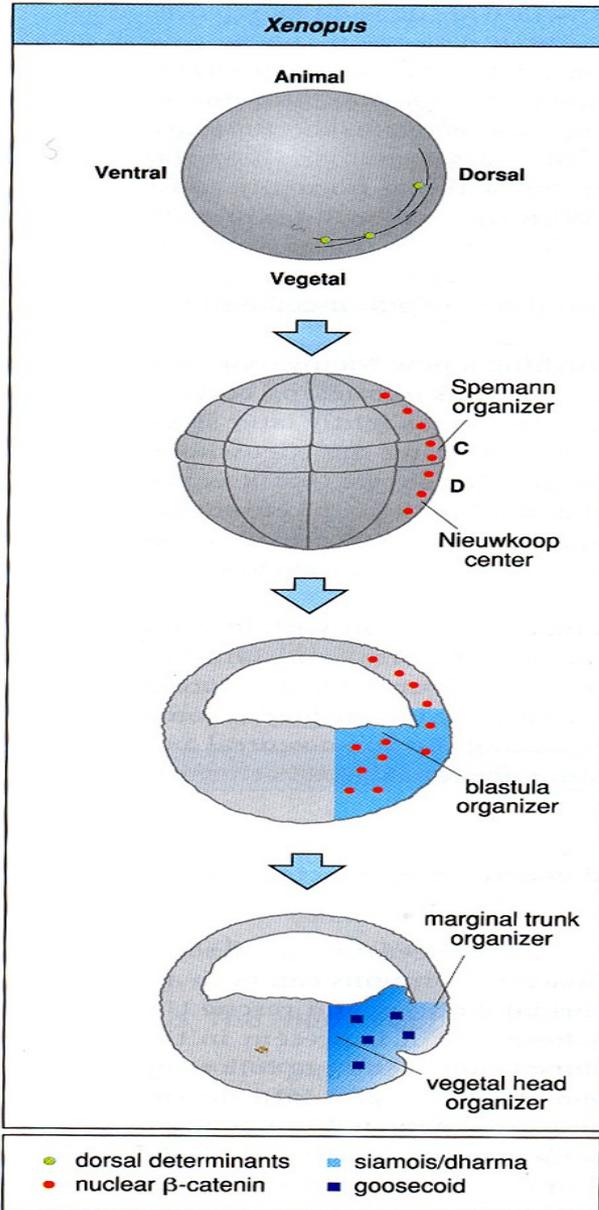
VegT RNA injection into vegetal/ventral blastomeres can induce secondary axis via induction of dorsal fate.....



I – primary axis
 II - secondary axis
 nt – neural tube
 nc – notochord
 green – muscles
 arrow – ectopic auditory vesicles



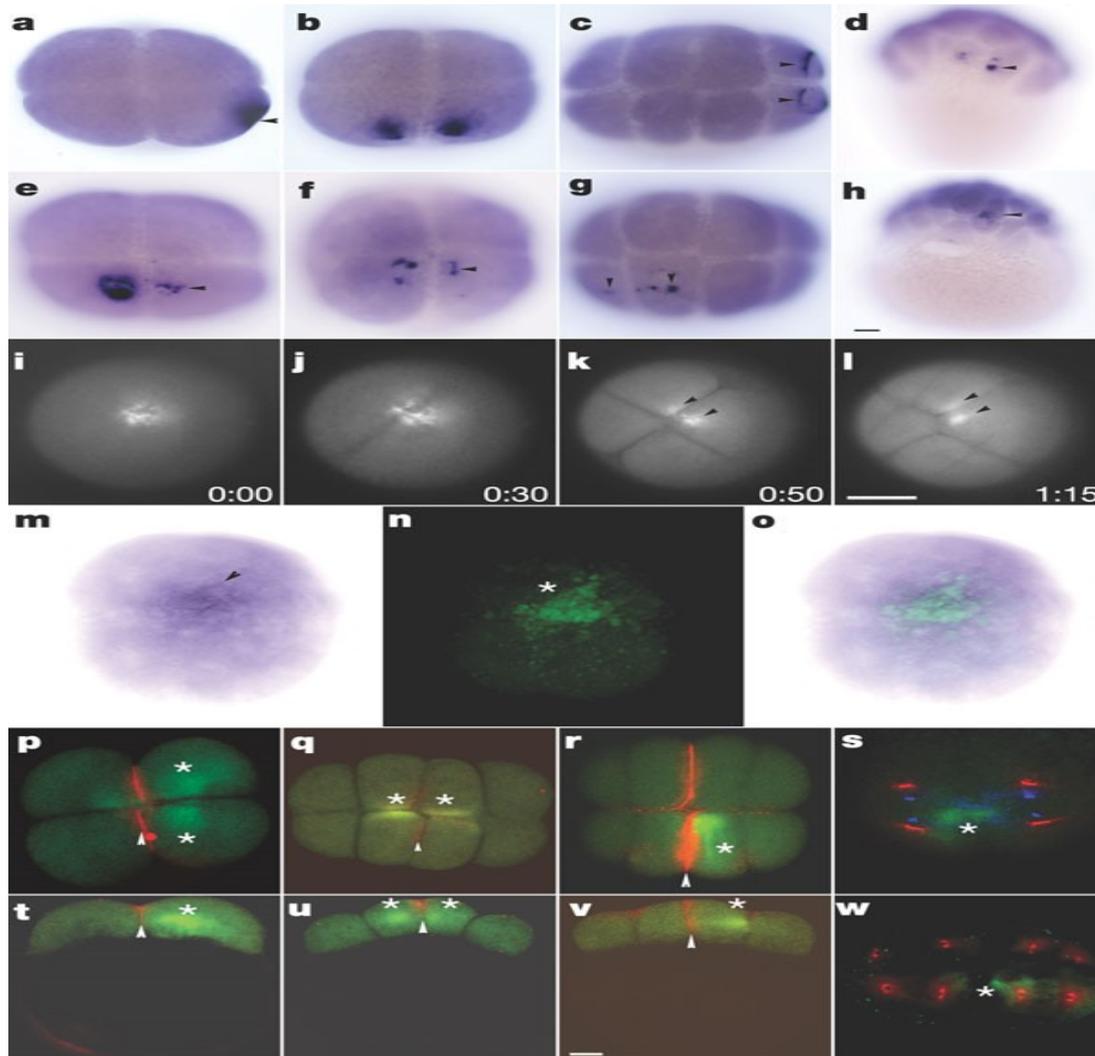
.....by activation of Xwnt8/ β -catenin pathway

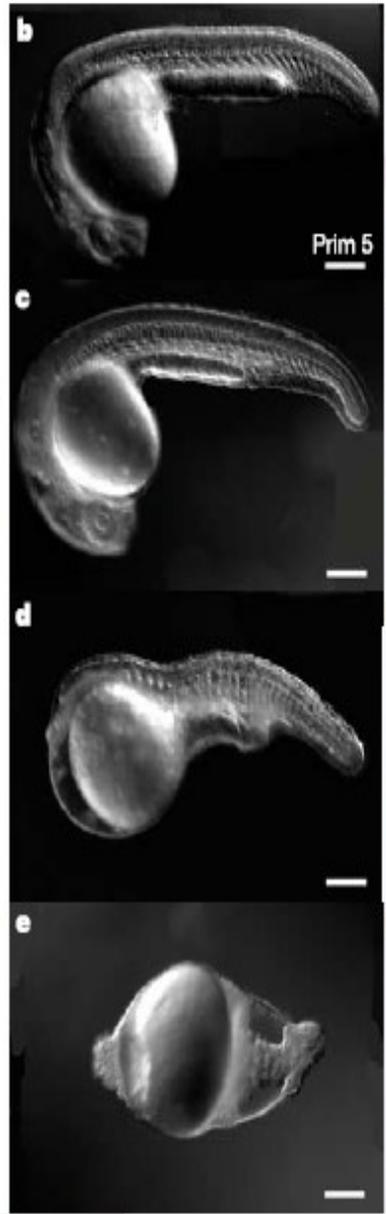
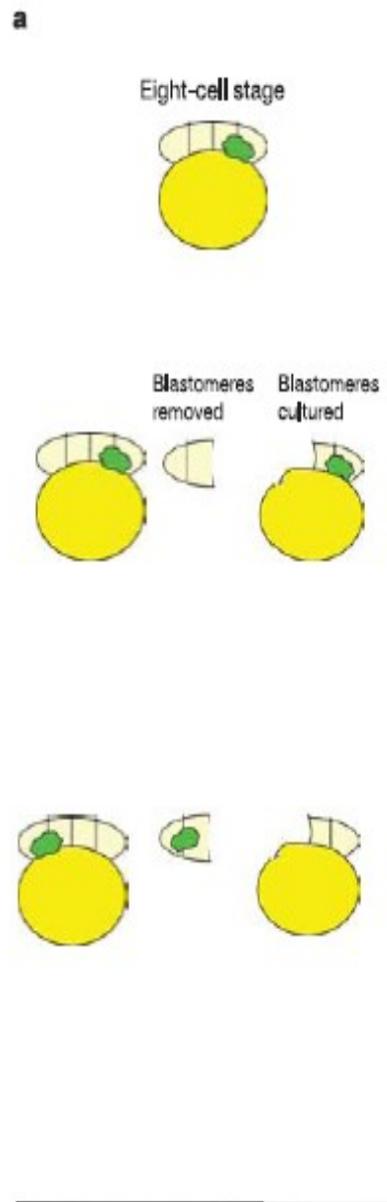


The zebrafish dorsal axis is apparent at the four-cell stage

Aniket V. Gore^{1,2}, Shingo Maegawa³, Albert Cheong¹, Patrick C. Gilligan¹, Eric S. Weinberg³
& Karuna Sampath^{1,2}

Squint – nodal-related morphogen important for establishment of dorsoventral axis





axial midline,
prechordal plate mesoderm

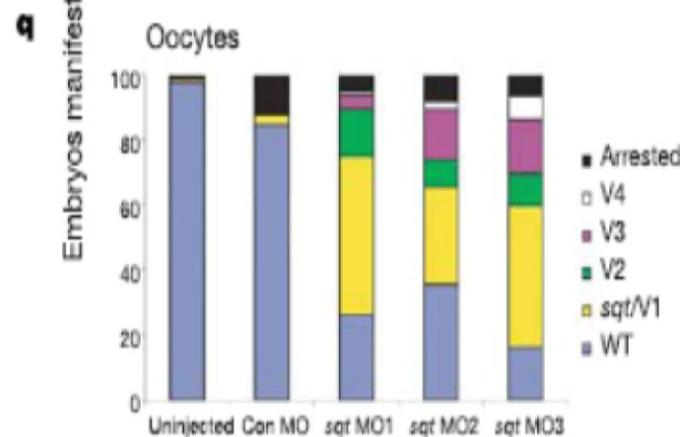
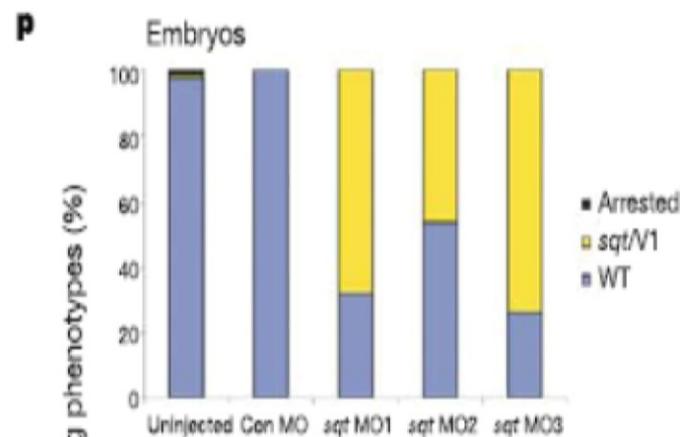
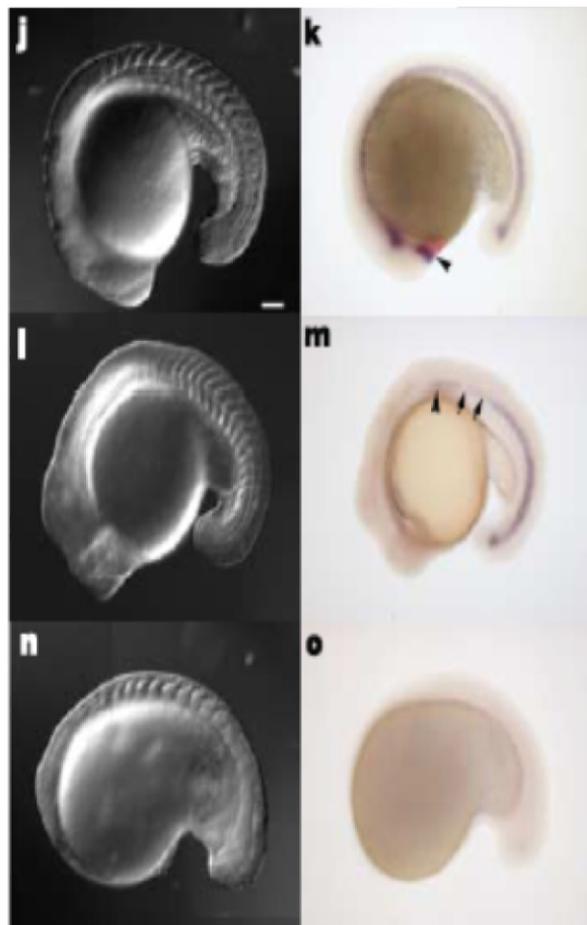


Figure 3 | Removal of cells with *sqt* RNA from embryos or injection of *sqt* morpholinos into oocytes leads to loss of dorsal structures. **a**, Schematic representation of eight-cell embryos to show removal of blastomeres with Alexa-488-labelled *lacZ:sqt* 3' UTR RNA (green, *sqt*⁺) or control removal of cells without *sqt* (*sqt*⁻). **b-e**, Live embryos at 24 h showing wild-type (WT) phenotypes in unmanipulated control (**b**), *sqt*⁻ cell removed (**c**), or *sqt*⁺ removed (**d, e**) embryos. **f-i**, Expression of *shh* (purple) and *hgg* (red) in unmanipulated (**f**), control *sqt*⁻ removed (**g**) or *sqt*⁺ removed (**h, i**) embryos. Phenotypes range from mild ventralization, V2 (not shown), and

loss of anterior structures, V3 (**d, h**), to complete ventralization, V4 (**e, i**), on *sqt*⁺ cell removal. Injection of morpholinos (MOs) against *sqt* into oocytes (**n, o, q**) also results in loss of dorsal and anterior structures, in comparison with oocytes injected with control *sqt* mismatch morpholinos (**j, k, q**) or fertilized embryos injected with *sqt* morpholinos (**l, m, p**). **j, l, n**, Live embryos at 18 h; **k, m, o**, expression of *shh* and *hgg* in the same embryos. Arrowheads in **k** and **m** mark the anterior limit of *shh* expression, and arrows in **m** indicate gaps in *shh* expression. Lateral views with anterior to the left. Scale bars, 100 μ m (**b, j**) and 50 μ m (**c-e**).

Vg1 (TGF β family ligand)

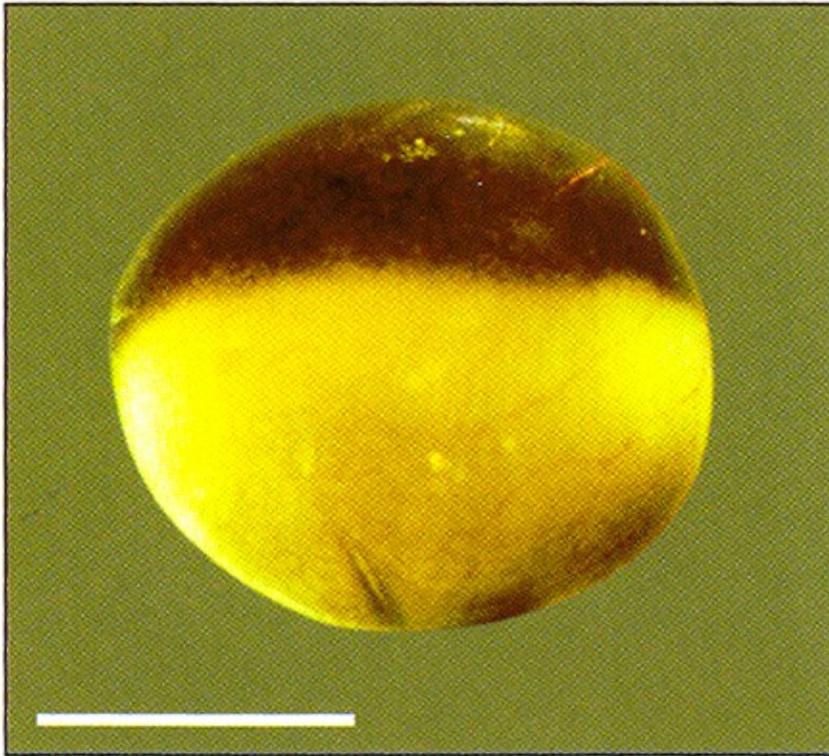


Fig. 2.4 The unfertilized egg of *Xenopus*. The surface of the animal half (top) is pigmented and the paler, vegetal half of the egg is heavy with yolk. Scale bar = 1 mm.

Photograph courtesy of J. Smith.

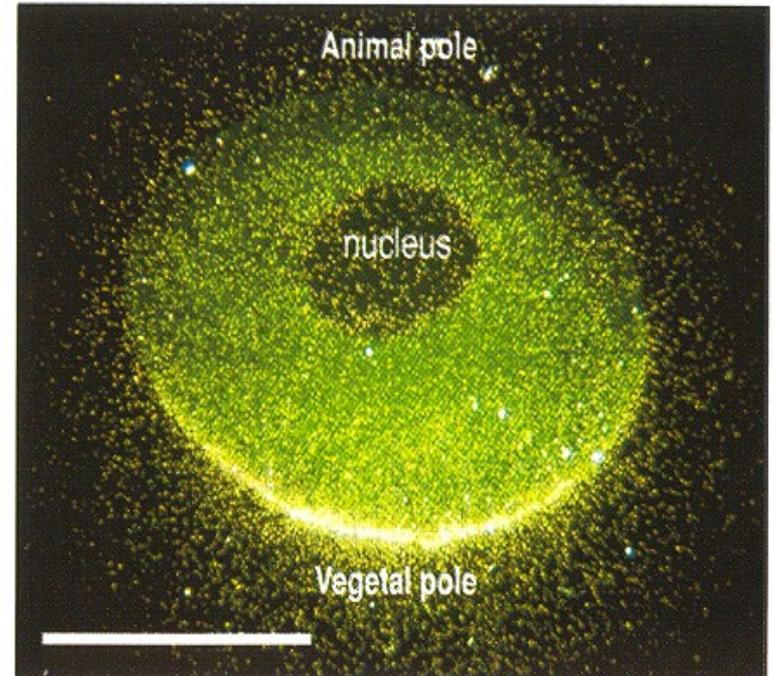
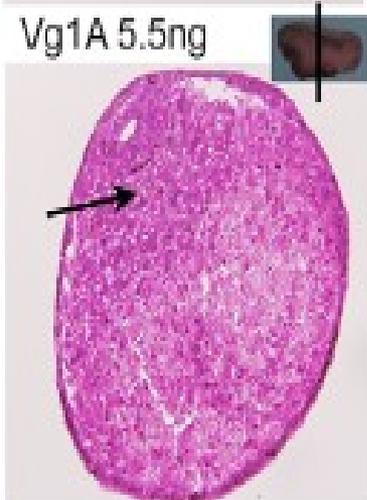
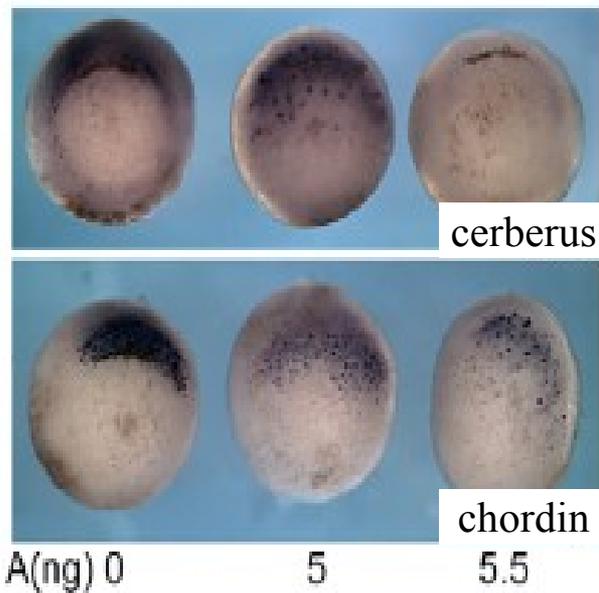
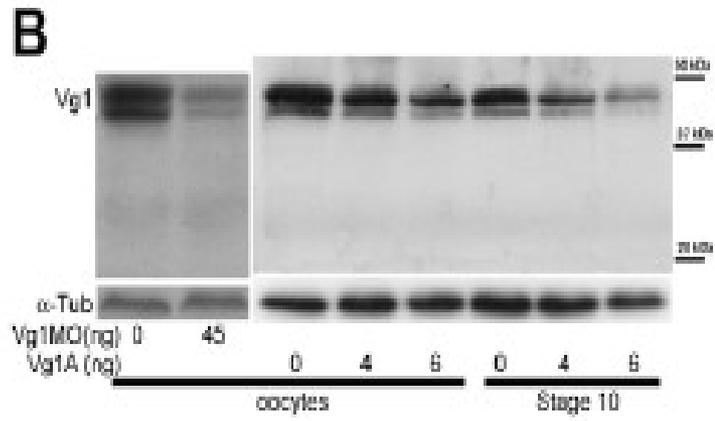
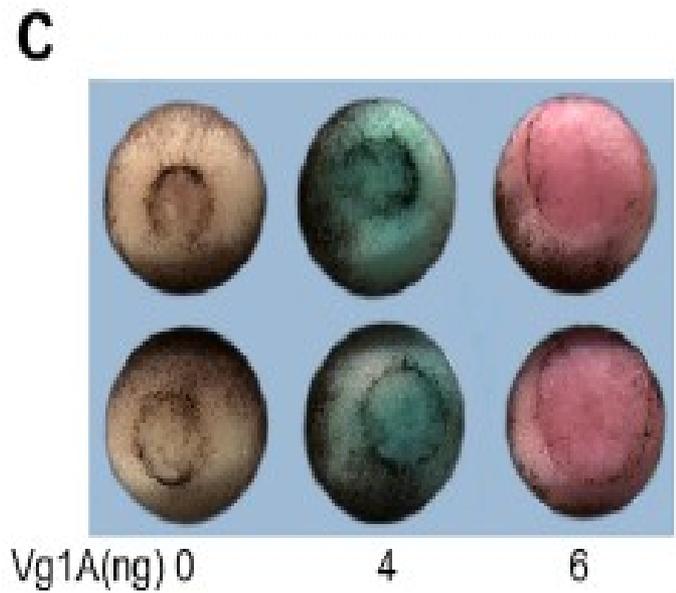
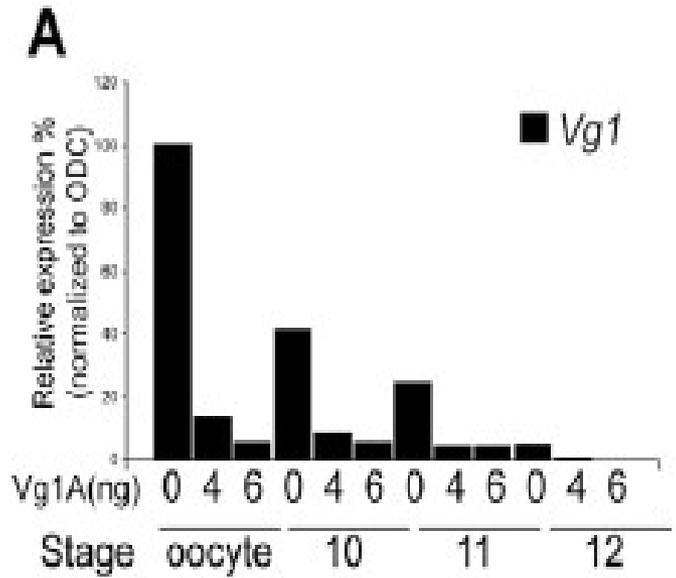


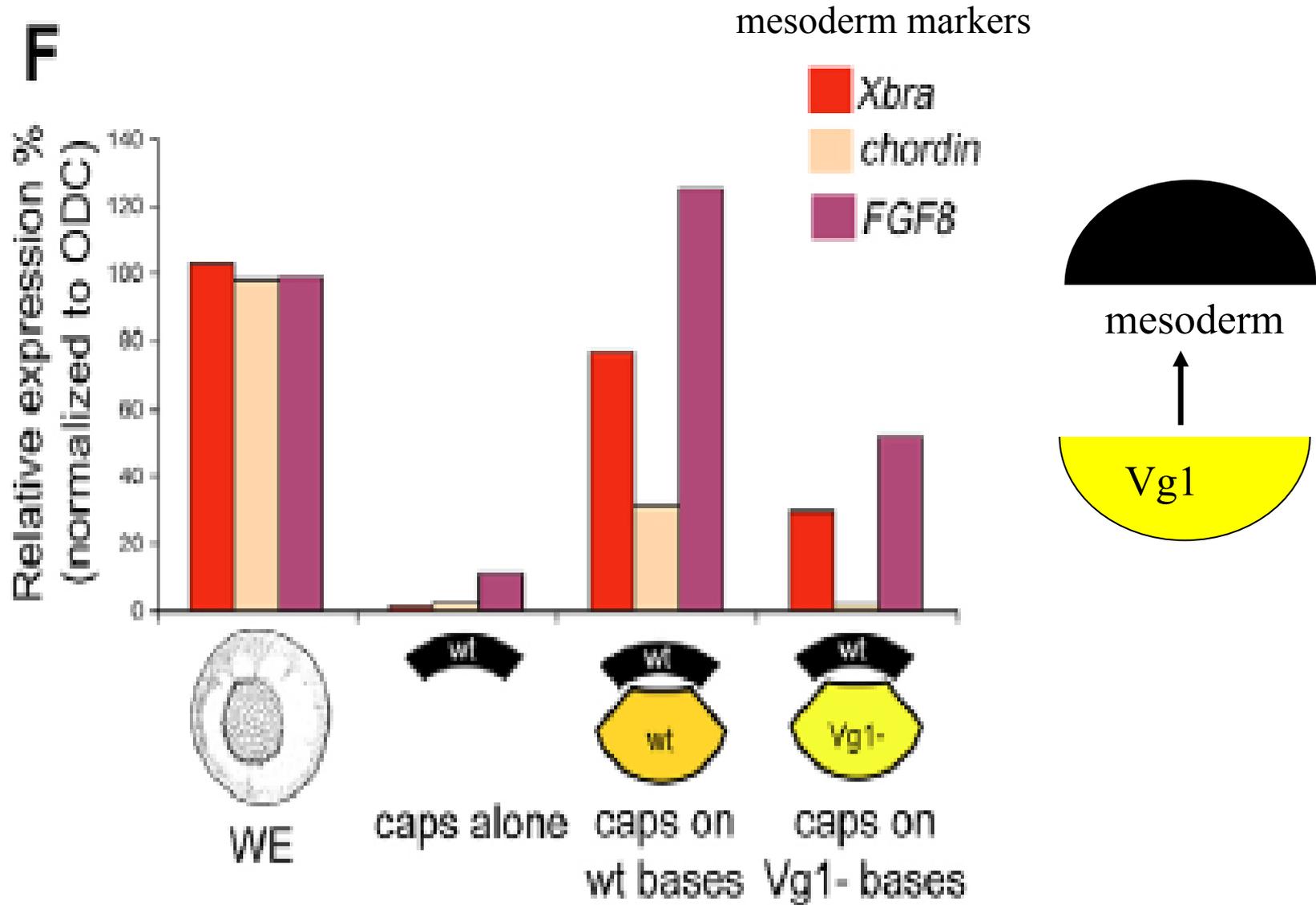
Fig. 3.2 Distribution of mRNA for the growth factor Vg-1 in the amphibian egg. *In situ* hybridization with a radioactive probe for maternal Vg-1 mRNA shows its localization (yellow) in the vegetal region. Scale bar = 1 mm.

Photograph courtesy of D. Melton.

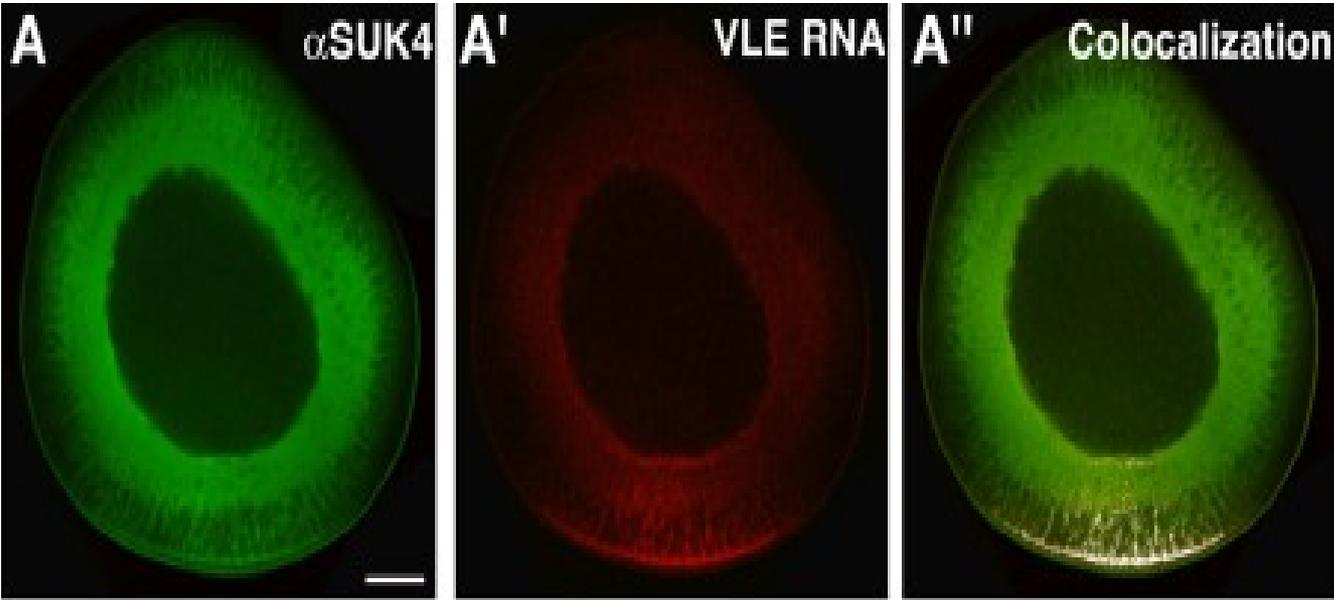
Vg1 depletion by morpholinos delays gastrulation and mesoderm induction with loss of head structures, absence of notochord and fusion of somites (arrow)



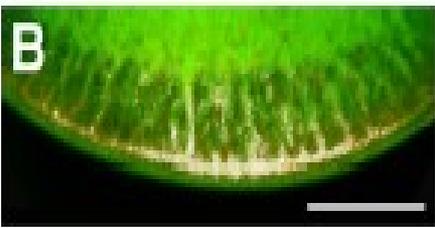
.....via loss of the induction of the mesodermal markers



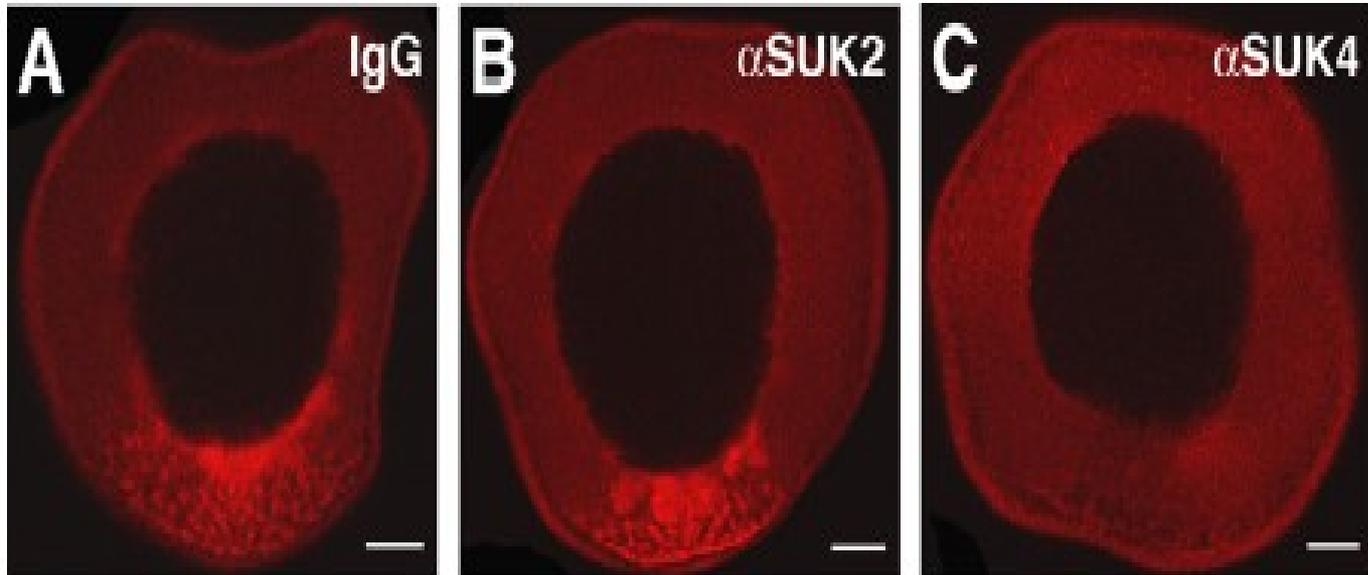
MECHANISMS OF INTRACELLULAR mRNA SORTING



VLE - Vg1
 SUK4- kinesin-1 heavy chain



total oocyte lysate



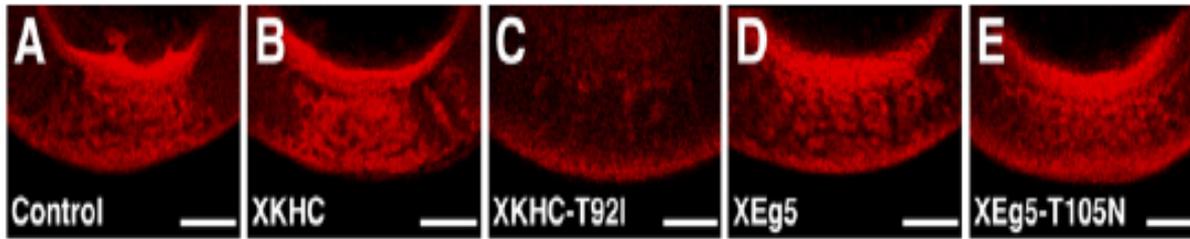
D

Injection	Localization (%)	<i>n</i>
IgG	100	62
α SUK2	102	36
α SUK4	52	53

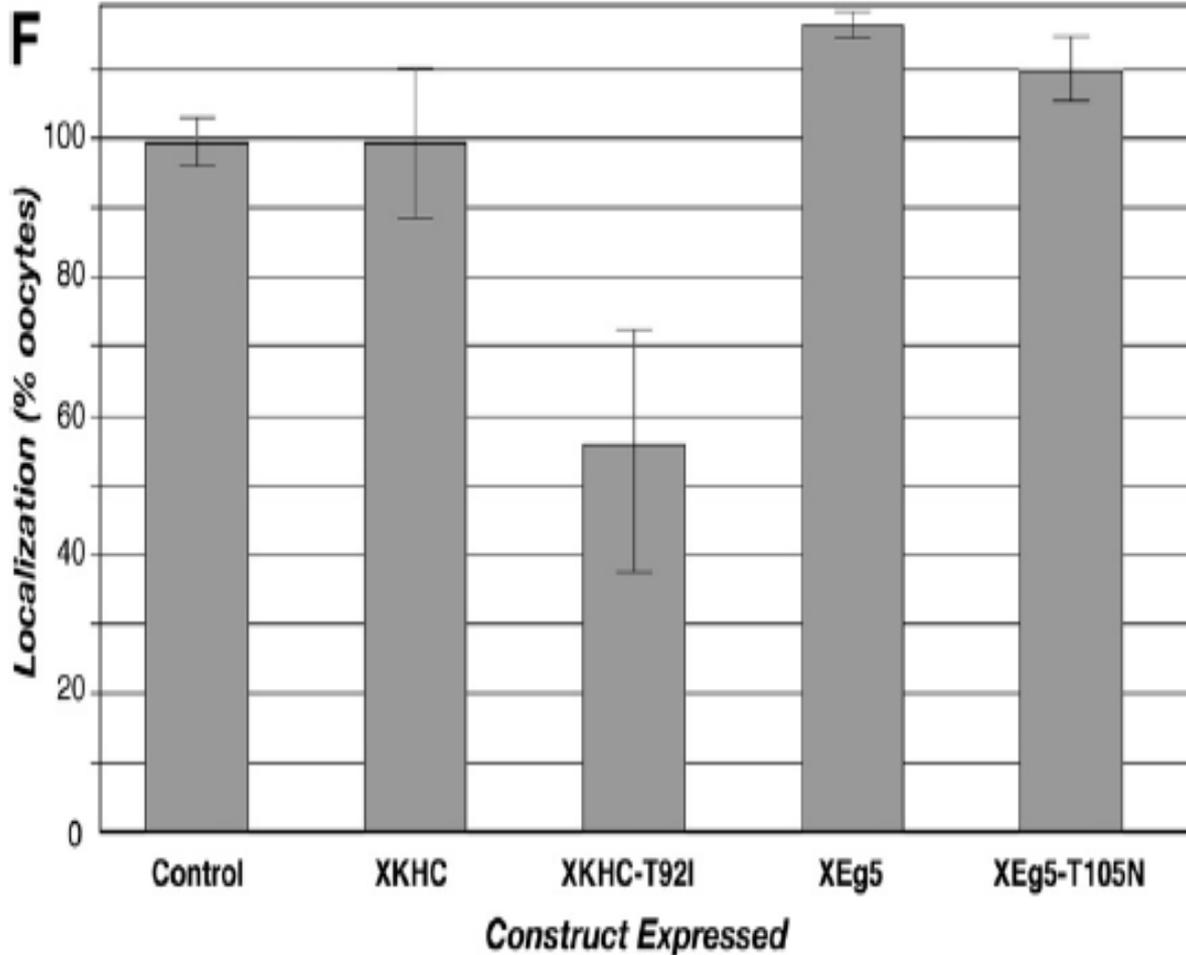
IgG – isotypic control
SUK2 –non-neutralizing Ab
SUK4 –neutralizing Ab



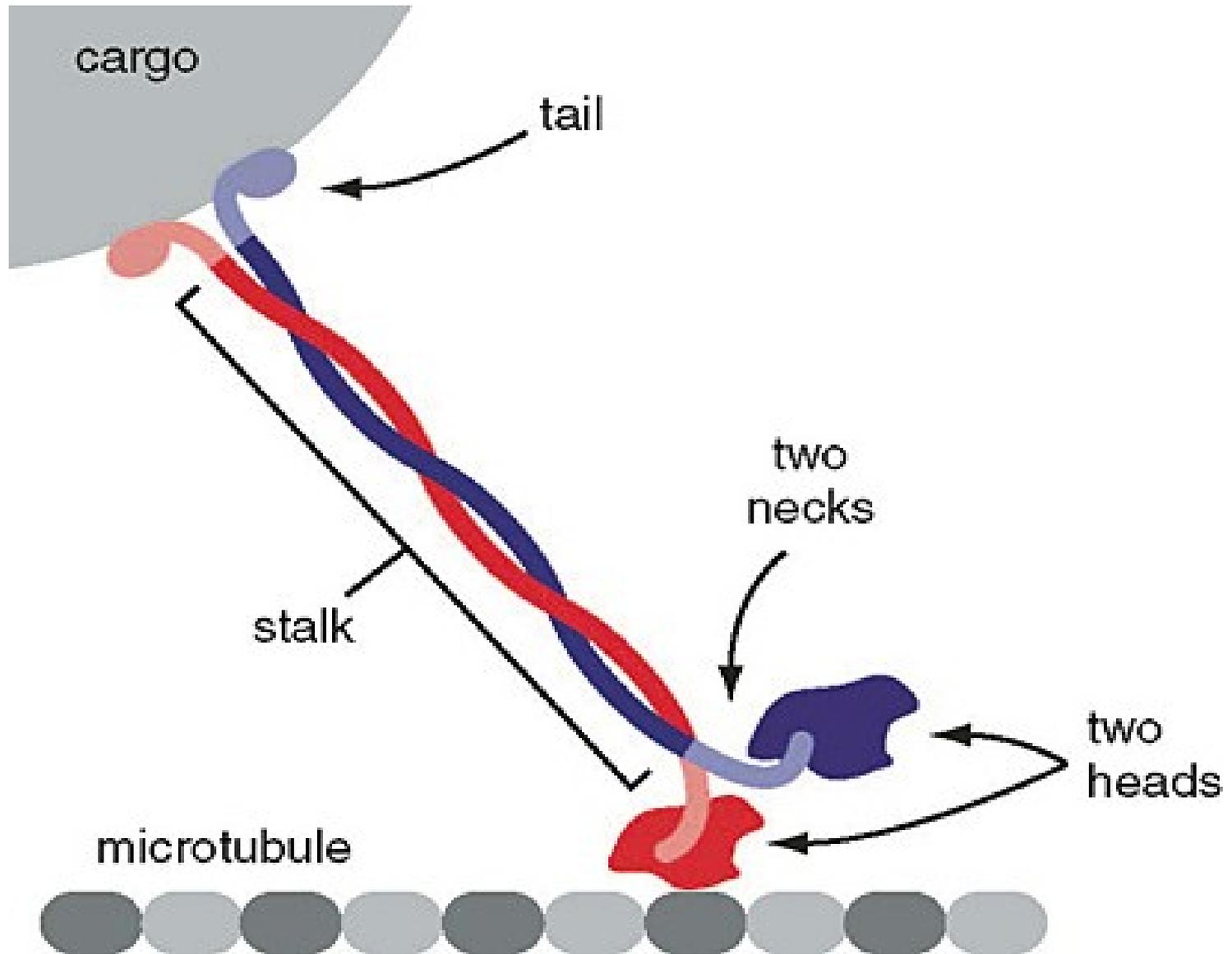
Vg1 mRNA localization



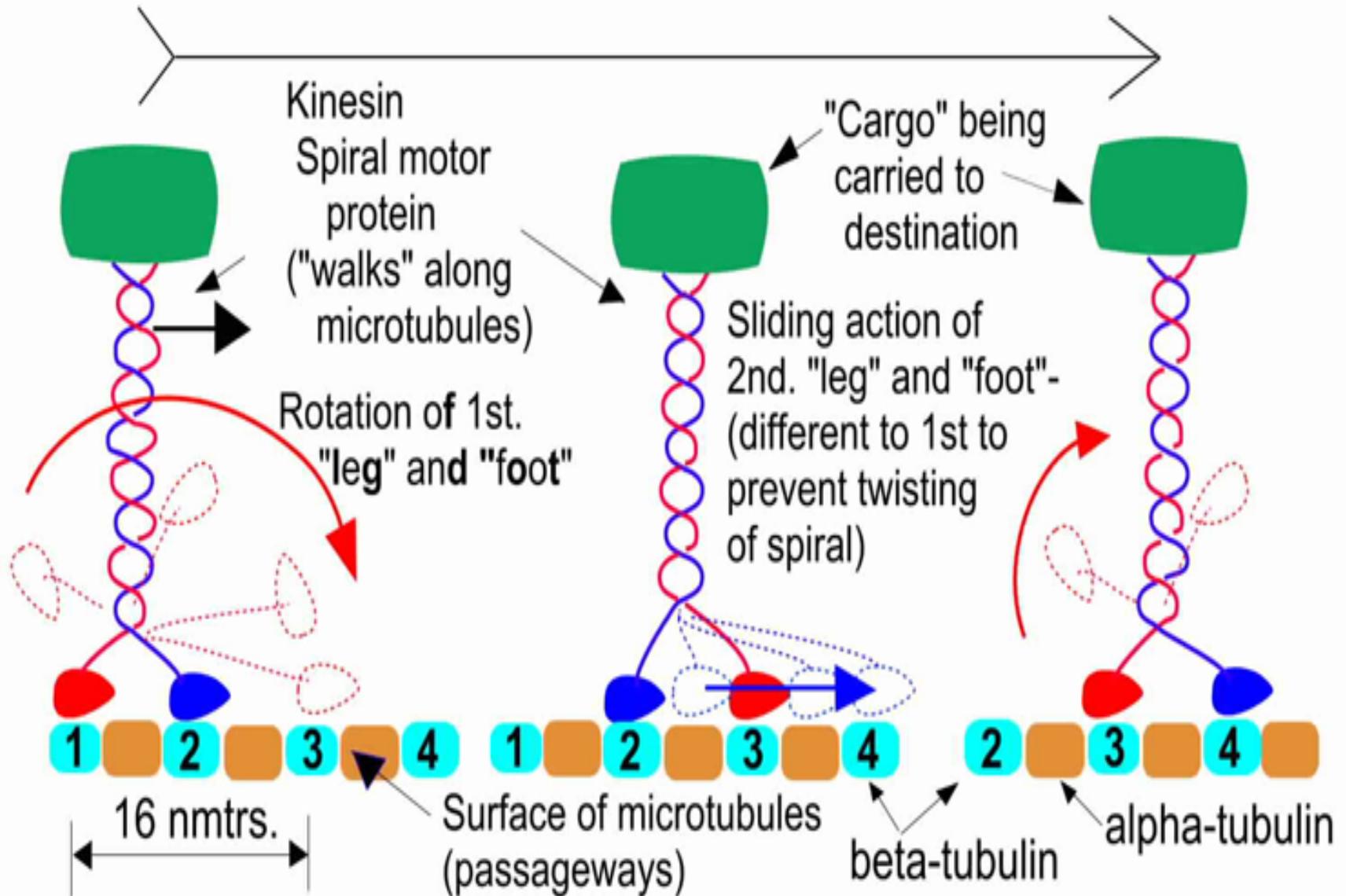
XKHC - kinesin 1
Red - injected Vg1 RNA
XKHC-T591 – rigor mutant
 (can not move – binds tight to
 microtubules)
XEg5-T105N – rigor mutant
 of kinesin unrelated to XKHC



KINESIN TRANSPORT



1 full cycle - uses 2 ATP - moves 16 nanometers



The (Ncr) Kinesin "walking" transportation of cell chemicals

